

## CLAIMS

1. Pair of oligonucleotides, for use as a set in the amplification of a target sequence of the genome of SARS

5 Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATTT TCTACAGGTG TTAACCTAGT  
AGCTGTACCG ACTGGTTATG TTGACACTGA AAATAACACA GAATTCACCA

10 GAGTTAATGC AAAACCTCCA CCAGGTGACC AGTTTAAACA TCTT,

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC  
AGAAGCTTCA CTT,

SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTTGGAAT GTCACGCATT  
GGCATGGAAG TCACACCTT, or

15 SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTTTA  
GTAGTGCTAT CCCCATGTGA TTTAATAGC TT,

or the complementary sequence thereof,

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

20 SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCTAAT ATGTTTATCA  
CCCGCGAAGA AGCTATTCGT CACGTTTCGTG CGTGGATTGG CTTTGATGT,

SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC  
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC  
AATCAACACC AATAGTGGTC CAGATGACCA AAT,

25 SEQ ID 26: CCAAACCTGTC ACTAAGAAAT CTGCTGCTGA GGCATCTAAA  
AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC  
ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT  
AACTAAACAG CACAAGTAGG TTTAGTTAAC TTTAATCTCA CATAGCAATC

30 TTTAATCAAT GT,

or the complementary sequence thereof.

2. Pair of oligonucleotides, according to claim 1, consisting essentially of:

a first oligonucleotide comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 3: TCCACCAGGT GACCAGTTTA AACATCTT,  
 5 SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,  
 SEQ ID 5: TACCTCTCCA GCTAGGATTT TCT,  
 SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,  
 SEQ ID 16: TAGGAACTGG CCCAGAAGCT TCACTT,  
 SEQ ID 24: TGCTCCAAGT GCCTCTGCAT TCTT,  
 10 SEQ ID 25: TTGGCATGGA AGTCACACCT T,  
 SEQ ID 32: TGCCTATATG GAAGAGCCC,  
 SEQ ID 33: TCCCCATGTG ATTTTAATAG CTT,  
 or the complementary sequence thereof, and

a second oligonucleotide comprising, at least a fragment  
 15 of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,  
 SED ID 7: GAAGCTATTC GTCACGTTTCG,  
 SEQ ID 8: TGCGTGGATT GGCTTTGATG T,  
 20 SEQ ID 18: AGGTTTACCC AATAATACTG CGT,  
 SEQ ID 19: AGATTCCCTC GAGGCCAGGG CGT,  
 SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,  
 SEQ ID 27: CCAAAGTGTG ACTAAGAAAT CTGCT,  
 SED ID 28: CTCAAGCATT TGGGAGACGT GGT,  
 25 SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,  
 SEQ ID 35: TACGATACAT AGTCTACTCT TGT,  
 SED ID 36: TAACTAAACA GCACAAGTAG GT,  
 SEQ ID 37: TAGCAATCTT TAATCAATGT,  
 or the complementary sequence thereof.

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3. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the replicase gene of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATTT TCTACAGGTG TTAAGTTAGT AGCTGTACCG  
ACTGGTTATG TTGACACTGA AAATAACACA GAATTCACCA GAGTTAATGC

5 AAAACCTCCA CCAGGTGACC AGTTTAAACA TCTT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCTAAT ATGTTTATCA

10 CCCGCGAAGA AGCTATTCGT CACGTTCGTG CGTGGATTGG CTTTGATGT, or the complementary sequence thereof.

4. Pair of oligonucleotides, according to claim 3, consisting essentially of:

15 a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 3: TCCACCAGGT GACCAGTTTA AACATCTT,

SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,

20 SEQ ID 5: TACCTCTCCA GCTAGGATTT TCT,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

25 SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,

SEQ ID 7: GAAGCTATTC GTCACGTTCG,

SEQ ID 8: TGC GTGGATT GGCTTTGATG T,

or the complementary sequence thereof.

30 5. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the gene encoding the Nucleocapsid protein of the genome of SARS Coronavirus, said pair consisting of:

35 a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC  
AGAAGCTTCA CTT, or the complementary sequence thereof, and  
a second oligonucleotide being 10-50 nucleotides in length  
and comprising at least a fragment of 10 nucleotides of:

5 SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC  
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC  
AATCAACACC AATAGTGGTC CAGATGACCA AAT, or the complementary  
sequence thereof.

10 6. Pair of oligonucleotides, according to claim 5,  
consisting essentially of:

a first oligonucleotide comprising at least a fragment of  
10 nucleotides of a sequence selected from the group consisting  
of:

15 SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,  
SEQ ID 16: TAGGAAGCTGG CCCAGAAGCT TCACTT,  
or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of  
10 nucleotides of a sequence selected from the group consisting  
20 of:

SEQ ID 18: AGGTTTACCC AATAATACTG CGT,  
SEQ ID 19: AGATTCCCTC GAGGCCAGGG CGT,  
SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,  
or the complementary sequence thereof.

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7. Pair of oligonucleotides, for use as a set in the  
amplification of a target sequence located within the gene  
encoding the Nucleocapsid protein of the genome of SARS  
Coronavirus, said pair consisting of:

30 a first oligonucleotide being 10-50 nucleotides in length  
and comprising at least a fragment of 10 nucleotides of:  
SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTTGGAAT GTCACGCATT  
GGCATGGAAG TCACACCTT, or the complementary sequence thereof,  
and

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a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 26: CCAAAGTCTG ACTAAGAAAT CTGCTGCTGA GGCATCTAAA  
 5 AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC  
 ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or the complementary sequence thereof.

8. Pair of oligonucleotides, according to claim 7,  
 10 consisting essentially of:

a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 24: TGCTCCAA GTGCCTCTGC ATTCTT,  
 15 SEQ ID 25: TTGGCATGGA AGTCACACCT T,  
 or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

20 SEQ ID 27: CCAAAGTCTG ACTAAGAAAT CTGCT,  
 SEQ ID 28: CTCAAGCATT TGGGAGACGT GGT,  
 SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,  
 or the complementary sequence thereof.

25 9. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the 3'-Non Coding Region (3'-NCR) of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length  
 30 and comprising at least a fragment of 10 nucleotides of:  
 SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTTTA  
 GTAGTGCTAT CCCCATGTGA TTTTAATAGC TT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length  
 35 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT  
 AACTAAACAG CACAAGTAGG TTTAGTTAAC TTAAATCTCA CATAGCAATC  
 TTAAATCAAT GT, or the complementary sequence thereof.

5 10. Pair of oligonucleotides, according to claim 9,  
 consisting essentially of:

a first oligonucleotide comprising at least a fragment of  
 10 nucleotides of a sequence selected from the group consisting  
 of:

10 SEQ ID 32: TGCCTATATG GAAGAGCCC,  
 SEQ ID 33: TCCCCATGTG ATTTAATAG CTT,  
 or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of  
 10 nucleotides of a sequence selected from the group consisting  
 15 of:

SEQ ID 35: TACGATACAT AGTCTACTCT TGT,  
 SEQ ID 36: TAACTAAACA GCACAAGTAG GT,  
 SEQ ID 37: TAGCAATCTT TAATCAATGT,  
 or the complementary sequence thereof.

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11. Pair of oligonucleotides, according to any of the  
 claims 1-10, wherein the first oligonucleotide is provided with  
 a promoter sequence recognized by a DNA dependent RNA  
 polymerase.

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12. Pair of oligonucleotides, according to claim 11,  
 wherein the first oligonucleotide consists essentially of the  
 sequence:

SEQ ID 9: aattctaata cgactcacta tagggAAGAT GTTTAAACTG  
 30 GTCACCTGGT GGA,  
 SEQ ID 10: aattctaata cgactcacta tagggAACAT AACCGATCGG  
 TACAGCTACT A,  
 SEQ ID 11: aattctaata cgactcacta tagggAGAAA ATCCTAGCTG  
 GAGAGGTA,  
 35 SEQ ID 39: aattctaata cgactcacta tagggAGAAG TACCATCTGG GGCTGA,



SEQ ID 40: aattctaata cgactcacta tagggAAGTG AAGCTTCTGG  
GCCAGTTCCT A,

SEQ ID 41: aattctaata cgactcacta tagggAAGAA TGCAGAGGCA  
CTTGGAGCA,

5 SEQ ID 42: aattctaata cgactcacta tagggAAGGT GTGACTTCCA TGCCAA,

SEQ ID 43: aattctaata cgactcacta tagggGGGCT CTTCATATA GGCA, or

SEQ ID 44: aattctaata cgactcacta tagggAAGCT ATTAAAATCA  
CATGGGGA.

10 13. Pair of oligonucleotides, according to any of the  
claims 1-12, wherein each oligonucleotide being 15-30  
nucleotides in length and comprising at least a fragment of 18  
nucleotides, and preferably being 18-26 nucleotides in length  
and comprising at least a fragment of 20 nucleotides.

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14. Oligonucleotide, for use as a probe to detect the  
amplified nucleic acid sequence resulting in the amplification  
of a target sequence located within the genome of SARS  
Coronavirus, said amplification being based on pair of  
20 oligonucleotides according to any of claims 1-13, said probe  
being 10-50 nucleotides in length and comprising at least a  
fragment of 10 nucleotides of:

SEQ ID 12: GTTCGTGCGT GGATTGGCTT TGATGTAGAG GGCTGTCATG  
CAACTAGAGA TGCTGT,

25 SEQ ID 21: GGCTACTACC GAAGAGCTAC CCGACGAGTT CGTGGTGGTG  
ACGGCAAAAT GAAAGAGCTC AGCCCCAGAT GGTACTTCTA TTACCTAGGA  
ACTGGCCCAG AAGCTTCACT TCCCTACGGC GCTAACAAAG AAGGCATCGT  
ATGGGTGCA ACTGAGGGAG CCTTGAATAC ACCCAAAGAC CACATTGGCA  
CCCGCAATCC TAATAACAAT GCTGCCACCG TGCTACAACCT TCCTCAAGGA  
30 ACAACATTGC CAAAAGGCTT CTACGCAGAG GGAAGCAGAG GCGGCAGTCA  
AGCCTCTTCT CGCTCCTCAT CACGTAGTCG CGGTAATTCA AGAAATTCAA  
CTCCTGGCAG CAGTAGGGGA AATTCTCCTG CTCGAATGGC TAGCGGAGGT  
GGTGAAACTG CCCTCGCGCT ATTGCTGCTA GACAGATTGA ACCAGCTTGA  
GAGCAAAGTT TCTGGTAAAG GCCAACAACA ACAAGGCCAA ACTGTCACTA  
35 AGAAATCTGC TGCTGAGGCA TCTAAAAGC CTCGCCAAA ACGTACTGCC

ACAAAACAGT ACAACGTCAC TCAAGCATTT GGGAGACGTG GTCCAGAACA  
 AACCCAAGGA AATTTCGGGG ACCAAGACCT AATCAGACAA,  
 SEQ ID 38: GCCACCACAT TTTCATCGAG GC,  
 or the complementary sequence thereof, provided with a  
 5 detectable label.

15. Oligonucleotide, according to claim 14, wherein the  
 probe is constituted by a molecular beacon, preferably  
 consisting of:

10 SEQ ID 13: 5'-[6-FAM]-ccatgggCTGTCATGCAACTAGAGATGCTGTcccatgg-  
 [DabSyl]-3',  
 SEQ ID 45: 5'-[6-FAM]-cgcgatGTTCGTGCGTGGATTGGCTTatcgcg-  
 [DabCyl]-3',  
 SEQ ID 22: 5'-[6-FAM]-ccatgggCTACTACCGAAGAGCTACCCGACGAcccatgg-  
 15 [DabSyl]-3',  
 SEQ ID 30: 5'-[6-FAM]-ccatggACCAAGACCTAATCAGACAAccatgg-  
 [DabSyl]-3',  
 SEQ ID 47: 5'-[6-FAM]-ccatgcGCCACCACATTTTCATCGAgcatgg-[DabSyl]-  
 3'.

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16. Use of an oligonucleotides' pair, according to any of  
 the claims 1-13, in a nucleic acid amplification reaction or as  
 a probe for the detection of SARS Coronavirus nucleic acid in a  
 sample.

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17. Method for the detection of SARS nucleic acid in a  
 sample wherein the sample is subjected to a nucleic acid  
 amplification reaction using a pair of oligonucleotides  
 according to any of the claims 1-13 and suitable amplification  
 30 reagents and the presence of any amplified nucleic acid is  
 detected.

18. Method according to claim 17, wherein the detection of  
 any amplified nucleic acid is carried out by reacting the  
 35 sample with an oligonucleotide according to claim 14 or 15



under suitable hybridization conditions and detecting the presence of the label in any hybrids formed between the amplified sequence and the probe.

- 5           19. Method according to claim 17, wherein the amplification technique used is a transcription based amplification technique, preferably the NASBA, and the first oligonucleotide is provided with a promoter sequence recognized by a DNA dependent RNA polymerase.

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20. Test kit for the detection of SARS Coronavirus in a sample comprising:

- 15           set of oligonucleotides according any of claims 1-13,  
          an oligonucleotide comprising a nucleic acid sequence substantially complementary to at least part of the amplified nucleic acid sequence, provided with a detectable label, according to claim 14 or 15, and  
          suitable amplification reagents.

- 20           21. Test kit according to claim 20, wherein suitable amplification reagents enable a transcription based amplification technique, preferably the NASBA.